The Tg.AC Workgroup Newsletter

Volume 1,Issue 1

March

Editor's Welcome

The Tg.AC Workgroup Newsletter is published by the Department of Toxicology and Safety Assessment, Boehringer Ingelheim Pharmaceuticals, Inc. as a means of communication for the HESI's Alternative to Carcinogenicity Testing Committee.

Letter and article submissions are welcome. Persons interested in contributing to the newsletter or receiving the newsletter should contact:

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On the Inside:

Welcome to the first issue of The Tg.AC Workgroup Newsletter, a publication sponsored by the Department of Toxicology and Safety Assessment, Boehringer Ingelheim Pharmaceuticals, Inc. The identification of carcinogens in the small rodent bioassay has played a prominent role in the risk assessment process. Most recent forums have discussed both the utility of the traditional chronic bioassay in the risk assessment process and the alternate strategies for obtaining more comprehensive profiles of toxicity for risk evaluation. Currently, much work is being done in the development of transgenic research animals as an expeditious, efficient alternative to the 2-year rodent bioassay used to identify chemical carcinogens and identify their health risk. Under the collaborative research program established by the Alternatives to Carcinogenicity Testing Technical Committee of ILSI Health and Environmental Sciences Institute over 40 research laboratories are currently developing scientific data to further understand and develop transgenic animals for the assessment of the carcinogenic potential of chemicals. The models that are being investigated include the Tg-rasH2 transgenic mouse, Tg.AC transgenic mouse (carrying the v-Ha-ras gene), p53+/- transgenic mouse, XPA repair-deficient transgenic mouse, the neonatal mouse and the SHE cell transformation assay. The results of the collaborative research program will be presented for public discussion tentatively in mid 1999. As results from this collaborative research emerge

issues regarding the procedures, evaluation and interpretation of data and application of models is continuously discussed. Evolution of this advancing knowledge is the consideration of the ICH guidance Testing for Carcinogenicity of Pharmaceuticals which allows for the use of a long-term rodent carcinogenicity study, plus one other supplementary study, e.g. transgenic rodents. This newsletter will provide a new forum to disseminate information regarding recent research, news, issues and events involving the Tg.AC mouse. I strongly encourage all the Tg.AC workgroup members to submit an update of their research to our newsletter since this would facilitate communication to all those involved with the Tg.AC model.

Sylvia Furst

National Toxicology Board of Scientific Counselors Meeting

February 5, 1998

A meeting was held at the NIEHS to discuss strategies for evaluation of transgenic models to assess carcinogenicity. Presentations were made by NIEHS and NTP staff describing on the most recent progress in studies of transgenic mouse models that appear to have promise in identifying potential carcinogens. Copies of the background material provided to the Board of Scientific Counselors can be obtained by contacting Dr. Larry Hart, National Toxicology Program, NIEHS, P.O. Box 12233, Research Triangle Park, NC 27709. His telephone number is 919-541-3971. The Board will provide guidance to the NTP in evaluation and validation of transgenic models, whether the scientific needs of regulatory agencies are being adequately addressed, how existing models can be best utilized, and what new models may be needed.

Attention

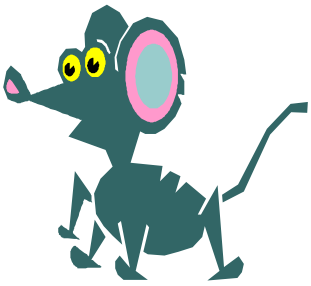
Important points to consider when conducting studies using Tg.AC animals have arisen as a result of a January 9, 1998 ILSI Tg.AC Workgroup Meeting.

- It has been reconfirmed that homozygous animals be used instead of hemizygous animals to reduce the effect of non-responsive phenotype on the results of the study (see article on non-responsive phenotype by Ray Tennant and Ray Stoll).
- Studies should always include a positive control group which receives phorbol 12-myristate 13-acetate (PMA) at 2.5 ug/mouse three times per week to ensure that a positive response is elicited from the Tg.AC model.
- At necropsy, any mass larger than 4 mm should be sampled and half of the mass snap-frozen for further gene expression analysis. A sample should also be taken from corresponding normal tissue from either the same animal or another animal.
- Tail snips should be obtained from all animals at the end of study for subsequent analysis for proof of responsiveness or unresponsiveness. It is suggested that 1 cm sections be taken and preferably three identical 1 cm sections, if possible.

Studies Currently Being Conducted at the NIEHS

by Ray Tennant, NIEHS

Current studies at the NIEHS focus on four principal areas. First, studies are being done to understand the factors in common between the discriminative response of the Tg.AC model to chemical carcinogens, tumor promoters and physical wounding. An association between time-dependent hypomethylation of the transgene and transgene expression has been shown. The GATA-3 transcription factor has been shown to bind to the transgene promoter and its role in the transcriptional regulation of transgene expression is under investigation. Second, studies are being directed at characterization of the cellular population of the follicular epithelium in which transgene expression is first found. Efforts are underway to develop other markers for the cellular population independent of transgene expression and to isolate and characterize the transgene expressing cell population. Third, studies concentrating on defining the mechanism of the non-responsive (NR) phenotype are underway in collaboration with Frank Sistare and colleagues at the FDA and at Boehringer Ingelheim. Fourth, is the assessment of Tg.AC bioassays for carcinogenicity. We continue studies to test the working hypothesis that the Tg.AC model can be utilized to identify mutagenic and non-mutagenic carcinogens that have trans-species (i.e., rat and mouse) carcinogenic potential and thereby reduce the influence of strain- or species-specific responses obtained in conventional bioassays. We have recently completed a prospective evaluation of eight substances that were undergoing two year conventional bioassays. The results of these studies are currently being compiled into a manuscript summarizing the results and implications of the study. A summary of the results of the study were presented to the National Toxicology Program report subcommittee of the Board of Scientific Counselors on December 10, 1997 and an Executive Summary of that presentation is available upon request.



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Studies Currently Being Conducted at Boehringer Ingelheim Pharmaceuticals, Inc.

by Sylvia Furst, BIPI

At Boehringer Ingelheim Pharmaceuticals, Inc., the Department of Toxicology and Safety Assessment is currently involved with the ILSI/HESI Collaborative Research Program on Alternative Models for Carcinogenicity Testing. Studies were recently completed which compared hemizygous and homozygous Tg.AC mice in order to determine the effect of zygosity on the resulting tumor incidence. Benzene or phorbol 12-myristate 13-acetate (PMA) were applied dermally for 20-26 weeks followed by a complete histopathological analysis. Both agents resulted in the formation of multiple drug induced papillomas, however, differences between the number of hemizygous and homozygous animals which responded to drug treatment were observed. An inter-laboratory comparison between hemizygous and homozygous mice treated at NIEHS and Boehringer Ingelheim found homozygous mice respond similarly between laboratories, however, a significant difference in response was noted between the laboratories with hemizygous mice. From these studies it was concluded that homozygous mice produce a more uniform response to carcinogens than hemizygous mice. This work will be presented at the 37th Annual Society of Toxicology Meeting in Seattle in March and full test and data have been submitted to Toxicologic Pathology for the July 98 issue. Other studies currently ongoing at our facility include

evaluation of sodium phenobarbital carcinogenicity in the Tg.AC mouse. This study includes three routes of administration (gavage, diet and dermal) as well as hemizygous and homozygous comparison. A study evaluating sulfisoxizole in the hemizygous and homozygous mouse via both oral and dermal administration is currently underway and is scheduled for termination in April. Evaluation of BHA and p-cresidine in the Tg.AC mouse via dermal and oral administration is also currently being conducted. In addition, a study evaluating dermal administration of glucocorticosteroids in the Tg.AC mouse is scheduled for necropsy the first week of March with a full histopathological evaluation to follow. Lastly, studies are currently being conducted to compare homozygous mice bred from a NIEHS colony with those bred from a Taconic colony using PMA. Results of these studies will be compiled and evaluated and presented to the ILSI Alternative to Carcinogenicity Committee in an effort to further evaluate the Tg.AC mouse as a model for carcinogenicity assessment.

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Boehringer Ingelheim

News and Views

Identification of a Non-Responsive (NR) Phenotype

by Chair and Co-Chair of the ILSI Tg.AC Committee, Ray Tennant and Ray Stoll

Research at the FDA along with collaborative studies between Boehringer-Ingelheim and NIEHS have led to the identification of a proportion of hemizygous animals from the Taconic Farms commercial colony that failed to respond to the positive control chemical, TPA. The exact proportion of animals varies from shipment to shipment and can pose some complexity in conducting further studies. Findings in the laboratory of Frank Sistare and his colleagues at the FDA, from efforts to genotype the non-responders (NR), are consistent with a genetic rearrangement and deletion in two copies of the transgene that are critical for its expression and appears to be diagnostic of the phenotype. Efforts are currently underway to identify and eliminate any such non-responsive animals from breeding stock. Further studies are being conducted at NIEHS to determine the origin of the phenotype and the mechanism by which it may have arisen. Until the problem is completely eliminated, the following recommendations would appear to be appropriate to insure that all proposed and ongoing studies can be completed satisfactorily. First, it is recommended that homozygous animals be used in lieu of hemizygous. Since the responsive phenotype appears to be dominant, the frequency of the NR phenotype is less among homozygous animals and there is therefore a reduced probability of an influence on experiments. Second, an aggressive dose of TPA should be used as a positive control (i.e., 2.5 ug three times per week). Third, at the end of the study tail clips should be taken and snap frozen from all animals so that they can be subsequently genotyped for responsiveness if judged necessary at some later time by the ILSI Tg.AC Committee. This latter procedure should eliminate any concerns about whether the absence of a response was due to lack of induction by the chemical or to the absence of a responsive phenotype.

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Ray Stoll at (203)798-5684

ILSI Basic Protocol for Tg.AC

Design of Study:

Group	No. of Animals
Vehicle Control (Acetone)	15 males and 15 Females[a]
Low Dose[c]	15 males and 15 Females[a]
Mid Dose[c]	15 males and 15 Females[a]
High Dose[c]	15 males and 15 Females[a]
Positive Control (TPA Phorbol Ester in acetone)[b]	15 males and 15 Females[a]

a Singly housed males and females
b 2.5 µg 3 times per week
c 7 days/week

Age of Animal: Homozygote 7 - 9 weeks old
Route: Dermal (volume 200 ul)
Vehicle: Acetone
Duration of Treatment: 26 weeks dosing

Clinical Observations & Body Weights: Mass palpations and clinical observations weekly; monthly body weights.

Necropsy: Gross necropsy: Gross lesions and approximately 40 tissues (includes masses) will be fixed in neutral buffered formalin. Any mass 4 mm diameter or greater, quick freeze ½ of mass.

Histopathology: A complete set of 18-20 tissues on all animals on study. As an alternative, a complete set of tissues minimally on negative Control and High dose; limited tissues on Low, Mid and Positive control.

Dose Level Selection: Bioassay MTD dose in mouse divided by 40 (average mouse weighs 25 grams divided into 1000 gm) to obtain ug or mg of compound to be applied dermally.

If no bioassay available, perform a dose range finding study to establish highest dose level applied dermally to induce erythema of the skin or signs of toxicity slightly below a life threatening dose level.

Check out the NTP Transgenic Web site!
http://ntp-server.niehs.nih.gov/Main_Page/transgen/Transgen_default.html

Articles of Interest

Cannon, R.E., Spalding, J.W., Trempus, C.S., Szczesniak, C.J., Virgil, K.M., Humble, M.C., and Tennant, R.W. (1997) Kinetics of wound-induced v-Ha-ras transgene expression and papilloma development in transgenic Tg.AC mice. Molecular Carcinogenesis 20(1): 108-114.

Tice, R.R., Nylander-French, L.A., and French, J.E. (1997) Absence of systemic in vivo genotoxicity after dermal exposure to ethyl acrylate and tripropylene glycol diacrylate in Tg.AC (v-Ha-ras) mice. Environmental and Molecular Mutagenesis 29(3): 240-249.

Trempus, C.S., Haseman, J.K., and Tennant, R.W. (1997) Decreases in phorbol ester-induced papilloma development in v-Ha-ras transgenic Tg.AC mice during reduced gene dosage of bcl-2. Molecular Carcinogenesis 20(1): 68-77.

Upcoming Meetings and Events

☑ Environmental Mutagen Society, 29th Annual Meeting
Disneyland Hotel
Anaheim, CA

March 21-26, 1998
Transgenic Animals Mini-Meeting held on March 24, 1998

☑ 1st Annual Meeting on Rodent Models in Modern Risk Assessment
The Jackson Laboratory
Bar Harbor, Maine

September 8-12, 1998